Implantation and Placentation in the dasyurid marsupial, *Sminthopsis crassicaudata*

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Abstract

This research has been performed on several aspects of the reproductive biology of female, fat-tailed dunnarts (*Sminthopsis crassicaudata*) which are small insectivorous Australian dasyurid marsupials. The study aimed to investigate the origin of the shell membrane, the stage of embryonic development at which it is lost and implantation takes place, the morphological features of fetal-maternal cellular interactions at implantation and placentation.

During early development a mucoid layer is laid down around the embryo just after fertilization in the ampulla of the fallopian tube, which may contribute to the block to polyspermy and provide an early nutrient source for the embryo. A second acellular layer, the shell membrane, is deposited around the embryo about the time it reaches the uterus less than 24h after fertilization which may give support to the cleaving blastomeres when they adhere to the thin zona pellucida. The shell membrane (SM) also allows the passage of gases and nutrients from the uterine fluid to the pre-implantation embryo and is not shed until just prior to implantation which generally occurs about two-thirds through pregnancy.

To solve the controversy of the origin of the shell membrane polyclonal antibodies were raised against the shell membrane and mucoid. Indirect immuno-fluorescence, streptavidin/biotin immunoperoxidase cytochemistry, and immuno-gold labelling revealed that precursors of the tertiary egg membranes are secreted by the luminal epithelium of the ampulla, isthmus (including the crypts), utero-tubal junction and adjacent endometrial glands. A variety of histochemical stains differentiated between the sites of mucoid and shell membrane precursor secretion. The mucoid coat consists of neutral and acidic glycoproteins and is secreted by luminal epithelial cells of the oviduct. The utero-tubal junction did not stain with any of the glycoprotein methods nor did the shell membrane although it is eosinophilic and stained positively with the red cytoplasmic stain of Masson's trichrome. Therefore, it is concluded that shell membrane precursors are secreted by non-ciliated cells of the luminal epithelium of the utero-tubal junction, adjacent glands, and by scattered glands in the anterior region of the uterus but not by any cell population of the oviduct.

Ultrastructural investigations of embryos at different times during pre-implantation development have shown that the SM is not present until zygotes have entered the utero-tubal junction and that it first has a compact granular consistency. As it thins

during blastocyst expansion it becomes fibrous in texture with fibres oriented mainly in the plane of the membrane.

The shell membrane is lost when the embryo is at the early somite stage allowing the trophoblast to become intimately associated with the maternal uterine epithelium at this time. At implantation, in the trilaminar, or vascular, region of the yolk sac (TYS), trophoblast cells adjacent to the embryo form desmosomes with uterine epithelial cells and appear to fuse with them to form hybrid cells. Later, as the placenta develops, in the bilaminar, or avascular, yolk sac (BYS) multinucleate trophoblast giant cells (TGCs) from an annular region adjacent to the sinus terminalis, penetrate the maternal epithelium by intrusion, possibly also by fusing with it. TGCs send processes down between maternal epithelial cells, break their intercellular junctions, and then form hybrid junctions. Maternal epithelial cells appear to be pushed apart by invading TGCs, which then appear to pause at the basal lamina before migrating into the stroma. Desmosomes subsequently develop between TGCs and maternal stromal fibroblasts. In the TYS placenta trophoblast is not invasive but its microvilli and larger cell processes invaginate, and interdigitate with, the highly folded maternal epithelium.

As the placenta develops TGCs in the BYS, which erode and phagocytose the maternal epithelium, migrate towards, but do not invade, the maternal capillaries; thus an endotheliochorial placenta results. In the TYS the convoluted chorion follows the contours of the highly folded endometrial epithelium but does not erode it, thus an epitheliochorial placenta is formed. The ultrastructure of trophoblast and endoderm in both placental regions suggests steroid and peptide biosynthesis as mitochondria with tubular cristae and smooth and rough endoplasmic reticulae are common.

In conclusion, the shell membrane is present for about 9 days of the 13.5 day pegnancy after which implantation proceeds. The ultrastructure of implantation and placentation of the dunnart suggests that, despite the fact that the placenta is derived from the yolk sac, its development, in particular that of the trophoblast, has many similarities with that of the eutherian chorioallantoic placenta. Although this study has largely been of a structural nature the results form the basis on which a number of functional hypotheses can be made which can be tested in the future.